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H. Li
*Iowa State University*

Nader Deeb
*Iowa State University*

H. Zhou
*Iowa State University*

C. M. Ashwell
*United States Department of Agriculture*

Susan J. Lamont
*Iowa State University*, sjlamont@iastate.edu

# Chicken Quantitative Trait Loci for Growth and Body Composition Associated with the Very Low Density Apolipoprotein-II Gene[1]

H. Li,*† N. Deeb,*[2] H. Zhou,* C. M. Ashwell,‡[3] and S. J. Lamont*[4]

*Department of Animal Science, Iowa State University, Ames, Iowa 50011-3150; †College of Animal Science and Technology, Northeast Agricultural University, Harbin 150030, China; and ‡Agricultural Research Service, Livestock and Poultry Sciences Institute, Growth Biology Laboratory, US Department of Agriculture, Beltsville, Maryland 20705-2350

**ABSTRACT** Very low density apolipoprotein-II (apoVLDL-II) is a major constituent of very low density lipoprotein and is involved in lipid transportation in chickens. The current study was designed to investigate the associations of an apoVLDL-II gene polymorphism on chicken growth and body composition traits. The Iowa Growth and Composition Resource Population was established by crossing broiler sires with dams from 2 unrelated highly inbred lines (Leghorn and Fayoumi). The $F_1$ birds were intercrossed, within dam line, to produce 2 related $F_2$ populations. Body weight and body composition traits were measured in the $F_2$ population. Primers for the 5′-flanking region in apoVLDL-II were designed from database chicken genomic sequence. Single nucleotide polymorphisms (SNP) between parental lines were detected by DNA sequencing, and PCR-RFLP methods were then developed to genotype SNP in the $F_2$ population. There was no polymorphism in the 492 bp sequenced between broiler and Leghorn. The apoVLDL-II polymorphism between broiler and Fayoumi was associated with multiple traits of growth and body composition in the 148 male $F_2$ individuals, including BW, breast muscle weight, drumstick weight, and tibia length. This research suggests that apoVLDL-II or a tightly linked gene has broad effects on growth and development in the chicken.

(*Key words*: very low density apolipoprotein-II gene, body composition, chicken, growth, quantitative trait loci)

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## INTRODUCTION

The candidate gene approach is a powerful method for finding the QTL responsible for genetic variation in the traits of interest in agricultural animal species (Rothschild and Soller, 1997). Similar to other economically important traits, most chicken growth and fitness traits are controlled by multiple genes (Deeb and Lamont, 2002). Understanding the genetic control of growth in chickens will provide an opportunity for genetic enhancement of production performance and physiology. Molecular MAS may be required to increase selection efficiency and make further improvements in production performance. Genetic markers linked with QTL allow for direct selection on genotype (Lamont et al., 1996). The combination of traditional genetic selection and modern molecular methods may be preferred for breeding chickens in the future.

Very low density apolipoprotein-II (apoVLDL-II) is a major constituent of the very low density lipoprotein fraction of hen serum. It is a small phospholipid-binding protein (Jackson et al., 1977), synthesized in the liver, and ultimately deposited in the developing oocyte, where it forms part of the low density fraction of the yolk (Luskey et al., 1974; Chan et al., 1976). The function of apoVLDL-II is the transport of neutral lipids (triacylglycerol) in the form of very low density lipoprotein in the plasma. This protein binds to phospholipid and forms an outer polar shell surrounding the water-insoluble lipid core (Chan, 1983). The apoVLDL-II protein is also present and detectable in the plasma and liver of normal young cockerels (Lin and Chan, 1980, 1981; Blue and Williams, 1981; Chan, 1983).

To help elucidate the genetic control of growth of rapidly growing chickens, apoVLDL-II was examined as a candidate gene for growth and body composition



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[2]Present address: Sygen International, PO Box 348, 3033 Nashville Road, Franklin, KY 42134.
[3]Present address: Department of Poultry Science, North Carolina State University, Raleigh, NC 27695.
[4]To whom correspondence should be addressed: sjlamont@iastate.edu.

**Abbreviation Key:** apoVLDL-II = very low density apolipoprotein-II; BMC = bone mineral content; BMD = bone mineral density; BMW = breast muscle weight; DW = drumstick weight; SHL = shank length; SHR = shank weight to length ratio; SHW = shank weight; SNP = single nucleotide polymorphism; TBL = tibia length.

traits. The objectives of the present study were to identify single nucleotide polymorphisms (SNP) in the apoVLDL-II gene, develop PCR-RFLP methods to detect those DNA polymorphisms in $F_2$ resource populations, and evaluate associations between apoVLDL-II SNP and traits of growth and body composition.

## MATERIALS AND METHODS

### Experimental Populations and Management

The Iowa Growth and Composition Resource Population (IGCRP), established by crossing sires from a commercial broiler breeder sire line with dams from 2 unrelated highly inbred lines (Leghorn and Fayoumi) was used (Deeb and Lamont, 2002). The 2 dam lines are >99% inbred (Zhou and Lamont, 1999). The $F_1$ birds were intercrossed within dam line to produce 2 related $F_2$ populations. Birds were raised in floor pens on deep-litter bedding and had access to feed and water ad libitum. Birds were fed commercial corn-soybean-based diets that met all NRC requirements (National Research Council, 1994). From hatch to 4 wk, birds received starter feed with 20% protein and 3% fat content (Purina Mills Meat Builder[5]). From 4 to 8 wk, birds were fed a grower ration[6] with 18% protein and 4.1% fat.

### Phenotypic Measurements

Body weight was measured at hatch and at 2-wk intervals up to 8 wk of age. Body composition traits were recorded at 8 wk of age. These measurements included breast muscle weight (BMW), drumstick weight (DW), shank weight (SHW), shank length (SHL), tibia length (TBL), abdominal fat weight, spleen weight, liver weight, and heart weight. Tibias were analyzed for bone mineral characteristics using the dual-energy x-ray absorptiometry technique (Haarbo et al., 1991; Slosman et al., 1992; Svendsen et al., 1993; Mitchell et al., 1997). Bone mineral measurements were carried out using a total-body dual-energy X-ray absorptiometry scanner.[7] The differential attenuation of low (38 keV) and high energy (70 keV) x-rays were measured using the small animal total body research software package in high resolution scan mode. Multiple tibias were scanned simultaneously, and subsequent image analysis was used to accurately measure the bone mineral content (BMC) of each tibia and the axial cross-sectional area for determination of bone mineral density (BMD) (BMD = BMC/area).

### Development of PCR-RFLP Assays

Genomic DNA was isolated from venous blood collected in EDTA. A PCR was carried out with 50 ng of genomic DNA from the 1 grandsire and 4 granddams (2 from each inbred line) to investigate sequence polymorphisms of the 5′ flanking region of the apoVLDL-II gene. The PCR products were purified and recovered using a Microcon Centrifugal Filter.[8] Purified PCR products were sequenced by the Iowa State University DNA Sequence and Synthesis Facility. Sequences were analyzed using Sequencher 3.1.[9] Restriction enzyme sites in these sequences were detected by the MBCR (Molecular Biology Computational Resource) package.[10]

The apoVLDL-II 5′- flanking region PCR primers (5′CCT CTA TGA CAT GGT TGC CT 3′; 5′ ATG GGT TTG ACC CTG CTA TG 3′) were designed to amplify a 492-bp fragment by Oligo 5[11] according to chicken genomic sequence in the GenBank database (accession number V00448). The reaction conditions were 94°C for 3 min, 35 cycles of 94°C for 1 min, 58°C for 1 min, 72°C for 1 min, and an extension at 72°C for 10 min. The 25-uL reaction volume included 50 ng of template, 1× reaction buffer, 5 pmol of each primer, 0.16 m*M* dinucleotidetriphosphate (dNTP), 1.5 m*M* $MgCl_2$, and 1 U of *Taq* polymerase.[12]

### Screening $F_2$ Population for Restriction-Enzyme-Detectable SNP

A PCR of DNA from each male $F_2$ bird was performed using the conditions previously described. The PCR product was digested using 3 U of *Sfc*I[13] at 37°C overnight. The restriction digests were electrophoresed for 1.5 h at 100 V on a 2.0% agarose gel with ethidium bromide. Individual PCR-RFLP fragment sizes for the gene were determined by visualizing the band pattern under ultraviolet light.

### Statistical Analysis

Data were subjected to a 3-way ANOVA using JMP (SAS Institute, 2000) with genotype (G) as the fixed effect and dam (D) and hatch (H) as random effects according to the model

$$Y = \mu + G + D + H + e$$

where Y is the dependent variable, $\mu$ is population mean, and e is the random error. Dam and hatch were main factors in the experiment design. Dam and hatch were included in the model if their effects were $P < 0.20$ on a given trait. Otherwise, they were excluded from the final model. The interaction G by H was not significant for all traits and, therefore, was not included in the model. The interactions of D by G and D by H were not

[5]St. Louis, MO.
[6]Whiton Feeds, Perry, IA.
[7]Lunar DPX-L, Lunar Corporation, Madison, WI.
[8]Millipore Corporation, Bedford, MA.
[9]Gene Codes Corporation, Ann Arbor, MI.
[10]Biomedical Computing Facility, Baylor College of Medicine, Houston, TX.
[11]National Bioscience, Inc., Plymouth, MN.
[12]Promega Co, Madison, WI.
[13]New England Biolabs, Inc., Beverly, MA.

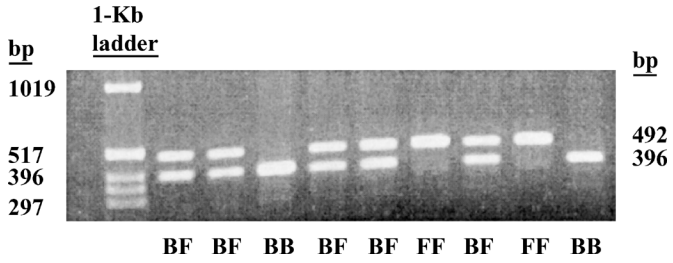


**FIGURE 1.** PCR-RFLP pattern for apoVLDL-II gene 5′-flanking region with *Sfc* I digestion. B = broiler allele; F = Fayoumi allele. The numbers listed on the left of the figure are sizes (bp) of molecular weight markers and those on the right are restriction-digest fragment sizes.

included due to missing data for combinations of dams with a low number of progeny. Significant differences between least squares means of the different genotypes were calculated using a contrast test. Significance was determined as $P < 0.05$, unless otherwise specified.

The percentage contribution of the gene to the total phenotypic variance was calculated as 100 times the ratio of the genotype sum of squares (SS) divided by the SS of all other components as were specified in the model

$$\frac{SS_G}{SS_G + SS_D + SS_H + SS_e} \times 100.$$

## RESULTS

### Sequence Variation and PCR-RFLP Analysis

The amplification product of the apoVLDL-II 5′-flanking region which included a portion of the first intron was 492 bp. Sequencing of multiple individuals of each parent line showed a G/A SNP between broiler and Fayoumi lines at base 634 (accession number V00448). The restriction enzyme *Sfc*I-digested PCR products had fragment sizes of 396 and 96 bp for the broiler line and 492 bp for the Fayoumi line. The grandsire (broiler) allele was designated as "B" and granddam allele as "F" for Fayoumi, respectively (Figure 1). One hundred forty-eight $F_2$ males from the broiler by Fayoumi cross were analyzed with the population representing progeny from one $F_1$ sire of Fayoumi cross. There was no sequence difference between the broiler and Leghorn population founders, and therefore genetic variation in this region of the *apoVLDL-II* gene could not be analyzed in this cross.

### Association and Effects of apoVLDL-II Gene SNP with Growth and Skeletal and Body Composition Traits

The apoVLDL-II polymorphism was predominantly related to growth, and muscle and skeletal traits (Table 1). There were significant associations between the apoVLDL-II polymorphism and BW at 2, 4, 6, and 8 wk of age, muscle traits (BMW, % BMW, and DW) and bone length traits (TBL) in $F_2$ offspring of the broiler by Fayoumi cross.

There were significantly higher BW2, BW4, BW6, and BW8 in $F_2$ birds that were homozygous for the apoVLDL-II broiler alleles than in those homozygous for the Fayoumi alleles (Table 2). With skeletal traits, there were significantly higher TBL, SHW, and shank weight to length ratio (SHR) but significantly lower percentages of BMD and TBL in $F_2$ birds with the apoVLDL-II broiler homozygous genotype than in birds of the Fayoumi homozygous genotype. There was no significant difference in BMD, SHL, percentage SHW, and percentage SHL between $F_2$ birds with the 2 homozygous apoVLDL-II genotypes. For body composition traits, there were significantly higher BMW, percentage BMW, DW, and percentage DW in $F_2$ birds that were homozygous for the broiler alleles than those homozygous for the Fayoumi alleles.

**TABLE 1. Effects (*P*-values) of very low density apolipoprotein-II (apoVLDL-II) polymorphism on chicken growth, and skeletal and body composition traits in broiler by Fayoumi $F_2$ population**

| Traits (units)[1,2] | apoVLDL-II |
|---|---|
| Growth | |
| BW2 (g) | 0.022 |
| BW4(g) | 0.003 |
| BW6 (g) | 0.019 |
| BW8 (g) | 0.045 |
| Skeletal measurements | |
| BMC (g) | NS[3] |
| BMD (g/cm$^2$) | 0.173 |
| TBL (mm) | 0.042 |
| SHL(cm) | 0.060 |
| SHW (g) | 0.103 |
| SHR (g/cm) | 0.089 |
| %BMC | NS |
| %BMD | 0.076 |
| %TBL | 0.076 |
| %SHW | 0.136 |
| %SHL | 0.084 |
| %SHR | NS |
| Body composition | |
| BMW (g) | 0.042 |
| DW (g) | 0.034 |
| AFW (g) | NS |
| SW (g) | NS |
| LW (g) | NS |
| HW (g) | NS |
| %BMW | 0.041 |
| %DW | 0.075 |
| %AFW | NS |
| %SW | NS |
| %LW | NS |
| %HW | NS |

[1]BW2, BW4, BW6, and BW8 = body weight at 2, 4, 6, and 8 wk, respectively; BMC = bone mineral content; BMD = bone (tibia) mineral density; TBL = tibia length; SHL = shank length; SHW = shank weight; SHR = shank weight to length ratio; BMW = breast muscle weight; DW = drumstick weight; AFW = abdominal fat weight; SW = spleen weight; LW = liver weight; and HW = heart weight.

[2]Percentage (%) indicates that traits are expressed as percentage of BW at 8 wk of age.

[3]Not significant at $P > 0.2$.

**TABLE 2. Effects of very low density apolipoprotein-II (apoVLDL-II) genotype on growth and skeletal and body composition (least squares means)**

| Traits (units)[1,2] | Genotype[3] BB | BF | FF |
|---|---|---|---|
| Growth | | | |
| BW2 (g) | 215.7[a] | 210.3[a] | 201.1[b] |
| BW4 (g) | 664.1[a] | 635.0[b] | 601.4[c] |
| BW6 (g) | 1152.0[a] | 1087.6[b] | 1056.6[b] |
| BW8 (g) | 1719.0[a] | 1650.8[ab] | 1597.1[b] |
| Skeletal measurements | | | |
| BMD (g/cm$^2$) | 0.2553[a] | 0.2484[b] | 0.2526[ab] |
| TBL (mm) | 113.31[a] | 113.20[a] | 110.96[b] |
| SHL(cm) | 8.987[ab] | 9.042[a] | 8.849[b] |
| SHW (g) | 34.98[a] | 33.54[ab] | 32.59[b] |
| SHR (g/cm) | 3.867[a] | 3.701[b] | 3.665[b] |
| %BMD | 0.0148[b] | 0.0153[ab] | 0.0157[a] |
| %TBL | 6.569[b] | 6.915[b] | 6.986[a] |
| %SHL | 0.5125[b] | 0.5370[a] | 0.5304[ab] |
| %SHW | 1.9832[ab] | 1.9862[a] | 1.9237[b] |
| Body composition | | | |
| BMW (g) | 220.2[a] | 206.5[b] | 200.7[b] |
| DW (g) | 80.48[a] | 76.82[b] | 73.96[b] |
| %BMW | 12.485[a] | 12.186[ab] | 11.865[b] |
| %DW | 4.557[a] | 4.546[a] | 4.437[b] |

[a–c]Means within a row with no common superscript differ significantly ($P < 0.05$).

[1]BW2, BW4, BW6, and BW8 = body weight at 2, 4, 6, and 8 wk, respectively; BMD = bone (tibia) mineral density; TBL = tibia length; SHL = shank length; SHW = shank weight; SHR = shank weight to length ratio; BMW = breast muscle weight; and DW = drumstick weight.

[2]Percentage (%) indicates that traits are expressed as percentage of BW at 8 wk of age.

[3]B = grandsire (broiler) allele; F = granddam (Fayoumi) allele.

### *Contribution of QTL Marked by ApoVLDL-II SNP to $F_2$ Phenotypic Variance*

The percentage of $F_2$ population phenotypic variance that was determined by apoVLDL-II gene SNP was calculated for all traits (Figure 2). The percentage contribution of apoVLDL-II to whole body growth rate traits (BW2, BW4, BW6, and BW8) ranged from 3.5 to 6.0%, to bone traits (BMD, TBL, SHL, SHW, SHR, %BMD, %TBL, %SHL, and %SHW) ranged from 1.6 to 3.5%, and to body composition traits (BMW, DW, %BMW, and %DW) ranged from 2.8 to 3.9%.

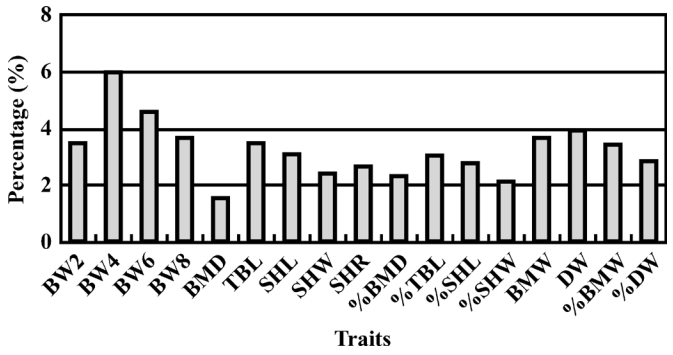


**FIGURE 2.** The contribution (%) of individual QTL to $F_2$ population phenotypic variance. BW2 = body weight at 2 wk; BMD = bone (tibia) mineral density; TBL = tibia length; SHL = shank length; SHW = shank weight; SHR = shank weight to length ratio; BMW = breast muscle weight; DW = drumstick weight. Percentage (%) on x-axis traits indicates that these traits are expressed as percentage of BW at 8 wk of age.

## DISCUSSION

### *Associations Between apoVLDL-II Gene Polymorphism and Traits*

The study of candidate genes is one of the primary methods to determine whether specific genes are related to economic traits in farm animals. The apoVLDL-II is a member of the avian yolk protein gene family (Wiskocil et al., 1980; Lazier et al., 1981; Noteborn et al., 1986; Evans et al., 1987; Binder et al., 1990). ApoVLDL-II was the first apolipoprotein structural gene purified by molecular cloning (Wieringa et al., 1979; Chan et al., 1980). The apoVLDL-II gene promoter contains many regulatory elements that have been previously related with gene expression (Van het Schip et al., 1983, 1986; Kok et al., 1985; Wijnholds et al., 1988, 1990; Iyer et al., 1991; Berkowitz and Evans, 1992; Ryan et al., 1994; Smidt et al., 1994). The apoVLDL-II gene also contains sequences within its first intron that increase transcription (Berkowitz and Evans, 1992). By using in vitro DNase I footprinting, 6 protein-binding sites were revealed throughout the first intron (Shuler et al., 1998). The current study found a G/A mutation at base 634 (GenBank accession no: V00448) in the first intron of the apoVLDL-II gene. Although this mutation is not in an identified protein-binding site, the polymorphism was associated with growth, gain, and skeleton and body composition traits of growing birds. In an $F_2$ cross of divergent lines, however, the linkage disequilibrium was substantial. The examined SNP in the first intron of the apoVLDL-

II gene might, therefore, have been closely linked with functional polymorphism in other regions of the ApoVLDL-II gene or other linked genes.

## Effects of ApoVLDL-II Genotypes on Traits

***BW and Gain.*** Growth is a comprehensive reflection of development of various parts of the body, and its final expression is the result of interaction among genetic, nutritional, and environmental factors (Scanes et al., 1984). Growth rate, especially in broiler chickens, has been intensely selected for more than a half century and will continue to be one of the most important economic traits in broiler chicken breeding programs. Growth is under complex genetic control, and uncovering the molecular mechanism of growth will contribute to more efficient selection for growth in broiler chickens (Deeb and Lamont, 2002). From the current results, an apoVLDL-II polymorphism was related to early BW, accounting for about 3.5 to 6% of the phenotypic variance in growth traits. The $F_2$ birds with 2 apoVLDL-II broiler alleles had higher BW at 2, 4, 6 and 8 wk of age. They also had greater average daily gains between 0 to 2, 2 to 4, and 6 to 8 wk of age than birds with 2 Fayoumi alleles (unpublished data). Given the high phenotypic correlation (correlation coefficients from 0.68 to 0.93) of BW at different ages in this population (data not shown), the consistent association of the SNP with BW at multiple ages was not unexpected. Comparison of mean values for all 3 genotype suggests that apoVLDL-II acts in an additive fashion on growth traits with the broiler allele contributing to greater BW. The allele association (broiler with heavier weight) is consistent with the selection history of broilers, emphasizing growth rate to market age. The present study thus identifies apoVLDL-II as a candidate gene of QTL for growth, which may be used to increase growth rate or market weight in molecular MAS programs.

***Skeleton.*** Leg problems are a serious issue in current broiler commercial production, resulting from the lack of coordination of development and growth between whole body mass and the skeleton system (Julian, 1998). Increasing bone strength and keeping proper skeletal proportions could increase bird welfare and production efficiency in breeding of heavy-bodied chickens. In the current study, BMC, BMD, TBL, SHL, and SHW were measured as indicators of bone strength and leg growth. The BMD and BMC have been used to evaluate skeleton strength and to investigate and predict osteoporosis in humans and mice (Arden et al., 1996; Klein et al., 1998; Devoto et al., 2001; Li et al., 2001). In the current study, the apoVLDL-II SNP between broiler and Fayoumi lines was associated with some skeletal and bone measurements, accounting for about 1.6 to 3.5% of the phenotypic variance in various skeletal traits. In the $F_2$ population, birds homozygous for the broiler apoVLDL-II allele had greater TBL, SHW, and SHR compared with Fayoumi homozygous birds. The broiler allele effect appeared to be additive for these 3 traits. The genotype effects in the current study were consistent with the observation in founder lines that the broiler line had greater TBL, SHW, and SHR than Fayoumi (Deeb and Lamont, 2002). The $F_2$ birds with the broiler homozygous apoVLDL-II genotype had a lower %BMD and %TBL than birds with homozygous Fayoumi genotype. Birds with the apoVLDL-II-BB genotype had a higher BW at 8 wk of age, and so it was not unexpected that these birds had lower %BMD and %TBL, which was consistent with the measurements on broiler and Fayoumi founder lines that the broiler line was lower on the 2 bone-related percentage traits than the Fayoumi line. This result suggested that the bone strength of apoVLDL-II-BB genotype birds was comparatively poor than that of apoVLDL-II-FF genotype ones. The results, therefore, pointed to apoVLDL-II as a good candidate gene of QTL that could be used to increase leg bone strength, as estimated by %BMD, in chickens.

***Breast and Leg Muscle.*** Increased BMW and DW are major goals in breeding of broiler chickens. In the current study, the apoVLDL-II SNP was associated with 8-wk BMW and DW, accounting for about 3.7 to 3.9% of the phenotypic variation. The birds with the broiler homozygous apoVLDL-II genotype had greater BMW and DW with 10 and 9% greater BMW and DW than the Fayoumi homozygotes, respectively. Comparison of mean values for all 3 genotype suggests that apoVLDL-II acted in an additive fashion on these 2 traits with the broiler allele contributing to greater BMW and DW. The specific apoVLDL-II alleles have effects in agreement with their line origin (i.e., broiler alleles associated with heavier phenotypes, as observed in the parental populations; Deeb and Lamont, 2002). The SNP genotype for apoVLDL-II was also associated ($P < 0.05$) with the important economic trait of breast muscle yield (%BMW). The mean breast muscle percentage of birds homozygous for the broiler allele was 12.5% compared with 11.9% for $F_2$ birds homozygous for the Fayoumi allele. Results from the current study identified apoVLDL-II as a potential candidate gene of a QTL useful for selection to increase breast and leg muscle yield of the chicken.

In summary, commercial breeding programs of broiler chickens have become more complex and challenging because so many objectives need to be simultaneously considered to reduce production costs, maintain health, and improve product quality. Breeding goals must include increased growth rate, increased breast muscle yield, decreased abdominal fat, maintenance of good development and growth of the skeletal system, and overall fitness. The relationships of these traits are complex, and some of the traits are very difficult to measure. Therefore, molecular MAS can improve genetic selection programs. The results from the current study indicated that a SNP marker in the apoVLDL-II gene was associated with growth rate, skeletal development, and muscle weight and yield in chickens growing to market weight and is, therefore, a potential marker for use in molecular MAS programs.

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